STUDIES ON THE ALLELOPATHIC EFFECTS OF PHYTOCHEMICALS FROM SELECTED PLANTS

1. INTRODUCTION

In modern agriculture, natural plant communities may be replaced by a single crop species or weeds, which can be recognized to be an important cause of ecosystem degradation and biodiversity loss. Worldwide, enormous amount of chemicals are used as herbicides to manage weeds in order to improve the productivity of crops. The excessive and indiscriminate use of these chemicals can cause the development of herbicide-resistant weed biotypes and also environmental pollution. Those compounds were found to affect not only the target organisms, but also other aspects of the ecosystem like soil, water, food, non pest species, and humans that come in contact with the chemical. The problems of herbicide resistance, pollution and hazardous effects of synthetic chemicals on the non-target plants and organisms have led scientists to pursue and develop alternate methods such as use of natural plant products and their analogues as herbicides.

Plant cells contain an array of secondary metabolites which includes tannins, terpenoids, alkaloids, flavonoids, steroids, resins, fatty acids, gums, phenol compounds etc. These secondary metabolites which were considered as waste products at earlier times were found to possess a wide range of chemical, physical and biological activities. They can function as (i) competitive weapons against plant pathogens (ii) agents of symbiosis between microbes and plants, nematodes, insects, and higher animals (iii) sexual hormones (iv) differentiation effectors and (v) metal transporting agents [1, 2, 3]. Some of the secondary metabolites when released by plants

have been shown to affect neighboring plants. The phenomenon is called ***allelopathy*** and the chemicals released are called as ***allelochemicals*** [4].

This phenomenon of direct or indirect, beneficial or adverse effects of a plant on the other through the release of chemicals into the environment, was first defined by Molisch [5] followed by Rice [6]. The chemicals responsible for bringing about such effects known as *Allelochemicals* are also called as *Allelotoxins* or *s*imply *Phytotoxins*. Most allelochemicals are classified as secondary metabolites, but not all the secondary metabolites are allelochemicals. Use of ***allelopathy***, which involves a ***plant secondary metabolite*** (PSM) mediated (predominantly negative) ***plant-to-plant interaction***, can be exploited for the protection of crops. It can explain the interactions between organisms in the environment and also allows the explanation of phenomena such as succession, dominance, soil exhaustion and changes in soil productivity. Determination of the allelopathic potential of various plant species and of their allelopathic relationships could be used to protect crops against weeds [7].

For this purpose, one of the directions taken by allelopathy research is the discovery of new donor plants for allelopathic substances. Screening of fractions of plant extracts or leachates for their effects on seed germination of various plant species are frequently used to identify phytotoxic compounds. When a plant is found to possess allelopathic or any other biological property, it is necessary to isolate, purify and characterize the responsible compound present in the plant. The active compound or compounds must be isolated in an amount adequate for identification and for further characterization in bioassays. This identification of allelochemicals involved in allelopathy is essential for the development of organic herbicides.

Incorporating allelopathy into agricultural management may reduce the use of herbicides. The use of allelopathy could be either directly through utilizing natural allelopathic

interaction, particularly of crop plants, or by using allelochemicals as natural herbicides [8]. In modern agriculture, allelopathy has a great potential in improving crop productivity, genetic diversity, maintenance of ecosystem stability, nutrient cycling and nutrient conservation, weed control, disease management and pest control. Moreover, the secondary metabolites possessing allelopathic activity can act as models for the synthesis of new generations of herbicides that will be safe to the environment and offers one of the best biological means of disease control [9, 10].

2. AIM OF THE STUDY

Allelopathy which is a natural and an environment friendly technique can serve as a unique tool for weed control, increasing crop yields, decreasing the use of synthetic pesticides, and also to protect the environment. The main aim of the study is to identify a plant possessing allelopathic effect and to detect the active principle responsible for the effect. A further aim is to isolate and identify the active allelochemical from the most potent plant which will be helpful for developing a new natural herbicide. Natural products are environment friendly and can eliminate the damages to human health as well as to ecosystem.

3. OBJECTIVES OF THE STUDY

The main objective of the present study is to identify a plant possessing allelopathic potential, isolation, purification and characterization of active principle from the plant, testing the broad spectrum activities of the compound etc.

The following procedures will be conducted as part of the study.

1. Screening of plant for allelopathic effect by seed germination bioassay.
2. Selection of few plants with potent allelopathic effect.

3. Testing the allelopathic effect of various extracts of these selected plants on a variety of seeds representing various plant families.

4. Isolation of the active principles from effective plant by standard protocols.

5. Purification of the active principle by standard techniques.

6. Testing the weedicidal effects of purified compound under laboratory and field-conditions.

7. Determining the mechanism of action of the compound.

8. Evaluating the broad-spectrum activities of the purified compound which included anti-bacterial, anti-fungal, anti-protozoal, larvicidal effect and also its effect on slugs and Earth-worm.

7. Characterization of the active principle by standard techniques.

4. MATERIALS AND METHODS

4.1. Preliminary screening

64 plants belonging to different plant groups viz. creepers, climbers, herbs, shrubs and trees were screened for determining allelopathic effect using seed germination bioassay by petri-dish method. The receptor seeds used as representative seeds included *Cicer arietinum* Acc1 (Bengal gram),*Vigna radiata* (Green gram/Cheru payar), *Arachis hypogea* (Groundnut/ Nilakkadalai), *Pisum sativum* (Green peas) and *Cicer arietnum Acc2* (Kabul Chana). The details of materials used and techniques adopted during the course of investigation are described below.

a.Preparation of the Extracts using various solvents

The extract for testing allelopathic effect was prepared by following the procedure of Ahn and Chung [11]. Two hundred gram of matured fresh leaves were harvested, cleansed, cut into small chips and finely ground with mortar and pestle. The ground plant material was serially extracted

with 50 ml each of water, methanol and Petroleum benzine, filtered through cheese cloth to remove debris and finally filtered using Whatman No. 1 filter paper, evaporated to dryness and redissolved in 50ml of distilled water.

b. Germination test

Fifteen test seeds of each species with uniform size collected were surface sterilized with 1% sodium hypo chlorate for 20 minutes, rinsed with distilled water to remove excess of chemical, and blotted dry before being sowed. The sterilized seeds were evenly layered on sterile cotton soaked with 20 ml of extracts, placed in petri-plate, incubated at 28˚ C in dark for six days. A control plate with same amount of water instead of extract was kept was also maintained. Equal volume of distilled water was added in the dishes when moisture content of the cotton declined. Those seeds with visible radicle were considered germinated [12]. The counting of the germination of the seed were done daily from the second day onwards. Termination of the experiment was done after three consecutive recordings had shown no further variation. The whole experiment was repeated four times and the average values were expressed.

The 'Germination Capacity' or 'Maximum Germination Percentage' ($\mathbf{G_{T)}}$ was calculated by the following equation

$$\mathbf{G_T = N_T \times 100 / N}$$

where $\mathbf{N_T}$ represents portion of germinated seeds in each treatment for the final measurement, **N** represents total number of seeds used in the bioassay. Plant extracts were considered as having an allelopathic effect when they caused a 90% or more reduction in the growth of rootlets or seedlings [13].

4. ii) Determination of a potent allelopathic plant

In the second step, percentage inhibition of germination has been calculated using the equation

$$I=100-(E_2\times100/\ E_1)$$

Where, **I** represents Percentage inhibition, $\mathbf{E_1}$ represents response of Control plant and $\mathbf{E_2}$ represents response of treated plant [14].

5. RESULTS AND DISCUSSIONS

Among the 64 plants screened, only a few number exhibited inhibition of germination on the five receptor seeds, either with aqueous and methanolic extracts were selected, whereas others were discarded. Based on these criteria, eleven plants have been selected for further experiment. This included *Helianthus annus* (Sunflower), *Mangifera indica* (Mango tree), *Azadirachta indica* A.Juss (Neem)., *Manihot esculenta* Crantz. (Topioca plant), *Syzygium cumini* (L.) Skeels var. *Cumini* (Black plum*), Solanum torvum* Sw. (Devil's Fig), *Theobroma cacao*.L.(Cocoa Tree), *Artocarpus heterophyllus* (Jack fruit tree), *Tamarindus indica* L.(Tamarind tree), *Adenanthera pavonina* (Coral wood) *and Garcinia gummi-gutta* (L.) Robs. Var. *gummi-gutta* (Malabar Gamboge*).*

Among these eleven plants screened, the plant ***Garcinia gummi-gutta*** exhibited maximum allelopathic effect. The aqueous as well as the methanolic extract of the plant exhibited the activity. Aqueous extract exhibited the inhibitory effect in the range 62-72%, whereas methanolic extract exhibited the activity of 79-88%. The extruded radicle of the test seeds that exhibited a temporary germination were found to get decayed in the third day itself.

Since the plant, ***Garcinia gummi-gutta*** exhibited maximum allelopathic potential equally against the five test seeds used in the experiment, it has been selected for further studies

The effect of methanolic extracts of leaves, stem and roots of the plant *Garcinia gummi-gutta* on the germination and growth of five receptor plants were tested and found that the leaf extracts of the plant showed maximum inhibition. The allelopathic effect of varying concentrations (10%, 25%, 50% and 100%) of the methanolic extracts of leaves of *Garcinia gummi-gutta* showed that the extract showed considerable inhibition at concentrations above 25% and that the level of inhibition increased with an increase in the concentration of the extract. Higher extract concentrations (100%) exhibited maximum growth inhibitions. A time-dependent variation in the response of the receptor seeds upon treatment with the extract has been observed for six days. The percentage inhibition has been found to increase with increase in time. The results of the study indicate that the allelopathic effect of *Garcinia gummi-gutta* leaf extract is a progressive one that affects biological process of germination and growth in a gradual and carving manner. Before exploiting the allelopathic potential of the plant for making herbicides, it is needful to endure knowledge regarding its effect on grown plants in neighborhood. Thus a study has been performed to endure the effect of the plant on matured crop seedlings. For evaluating the impact, parameters like root length, shoot length, dry weight and fresh weight of the seedlings have been studied. Results revealed that the extract is not posing any harm to plants that have already germinated.

In order to isolate and purify the active principle from plant in pure form, soxhlet extraction method using organic solvents of different polarity has been performed followed by isolation and purification using column chromatographic techniques. The identity of the purified

fraction was determined by Qualitative chemical tests which revealed the presence of Flavonoids as active principle.

Weedicidal effect of the compound has been assayed which revealed that the plant is a strong weedicide. To determine the action of the compound in soil media, a pot experiment and field- studies have been performed. From these information, an idea regarding the extent of allelopathic activity of the compound under natural conditions has been obtained. An attempt has been made to study the mechanism by which the compound is exerting the inhibitory action on seeds. As part, amount of protein and Amylase activity were determined. Results revealed that the reduction in the protein and carbohydrate availability are paving the way for inhibition of seed germination.

Several varieties of crop plants can suffer from fungal, bacterial and various protozoan diseases that can reduce the yield or crop. Thus as part of determining the other biological activities of the compound, antibacterial effect, antifungal effect, antiprotozoal effect, larvicidal effects, effect of the compound on slugs and earth-worm has been performed. The results revealed that the compound does not possess a significant antibacterial effect, but it was found to possess antifungal, antiprotozoal and larvicidal effects. Among the bacterial species used, soil bacteria like Pseudomonas sp., which are useful for the growth of plants, were also included. Since antibacterial effect was found to be negligible for the flavonoids isolated from *Garcinia gummi-gutta*, the plant when applied as a herbicide will not be harmful those organisms residing in the soil. For testing the antifungal effect, phytopathogenic as well as animal pathogenic fungus were used. The results confirmed that the plant is an effective antifungal agent. Slugs are usually harmful to plants. The results obtained here in biological assays with these plant species clearly demonstrates that the plant presents a great pesticidal potential. Hence the plant can be used as a

promising biopesticide. Earthworms are a major component of the soil fauna which can improve the fertility of soil and are beneficial for the growth of plants. The application of chemical fertilizers, sprays, and dusts are believed to have a disastrous effect on Earthworm which when harmed can also affect the growth and activities of plants. Hence to evaluate the toxic effect of the compound on Earth worm, it is needful to test the effect of the compound before utilizing compound as a herbicide. A varying concentration of the compound when applied revealed that the compound is found toxic only at a maximum concentration. The present finding suggests that the use of the purified compound of *Garcinia gummi-gutta as* an herbicide will not be harmful to Earthworm residing in the soil.

In this work, for identifying the active principle present, the methanolic extracts of leaves of *Garcinia gummi-gutta* has been subjected to isolation and purification through a activity guided bioassay approach. The purified sample when analyzed through qualitative chemical analysis confirmed the presence of of Flavonoids which belongs to the family of phenols. These flavonoids when subjected to characterization procedure based on standard protocols have identified that the compound was found to possess a molecular formula, **2-(5'-carboxy-6'- hydroxy phenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one**, having similarity with that of Quercetin, a major flavonoid. The work suggests that the plant *Garcinia gummi-gutta* is an allelopathic plant and the compound isolated can be used as a potential herbicide.

6. CONCLUSION

The indiscriminate use of herbicides to manage weeds caused the development of herbicide-resistant weed biotypes, environmental pollution, damage to non-target plants and organisms etc. All the above mentioned threats led scientists to search for alternate methods such as natural plant products and their analogues as herbicides which are environment friendly and can also be

helpful to eliminate the damages to human health as well as other organisms. Thus the conclusion drawn from this study is that the plant, *Garcinia gummi-gutta* is a plant with promising allelopathic property. The active principle present in the plant were found to be flavonoids, belonging to the family of polyphenol group. The compound was also found to possess broad-spectrum activities. The impact of the compound on matured seedlings revealed that the compound will not be harmful to grown plants in the neighborhood. A compound when suggested as an herbicide, it is important to identify its effect on the soil biota, since the destruction of the soil biota can seriously harm the growth of plants. In the present study, the effect of the compound on Earth-worm showed that it is not harmful to such organisms. From all these findings, we can clearly conclude that the flavonoid compound, 2-(5'-carboxy-6'- hydroxy phenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one, isolated from *Garcinia gummi-gutta* can be used as a potent natural herbicide and can act as models for the synthesis of new herbicide that will be safe to the environment.

7. SUMMARY

The study was conducted to identify a plant possessing maximum allelopathic potential, which can be helpul for utilizing crop protection. For this purpose, 64 plants belonging to different plant groups were screened for allelopathic activity by seed germination bioassay. Among them, 11 plants were found to possess the activity. Of these, leaf extracts of the plant *Garcinia gummi-gutta* exhibited maximum allelopathic potential. Hence the plant has been selected for further studies. The isolation and purification of active principle from the plant and then, testing the broad spectrum activities of the compound has also been performed. The results revealed the presence of a flavonoid compound, 2-(5'-carboxy-6'- hydroxy phenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one, as the active principle. To confirm the toxicity of the compound in natural media

like soil, field-studies and pot experiments were done which also proved the herbicidal potential of the compound. It was also found to possess weedicidal potential as well. The studies on the broad-spectrum activities of the compound showed that it possesses antifungal, antiprotozoal and larvicidal effects, but with negligible effect on bacteria. The compound also can kill plant pests like slugs. It was also observed that the compound is not found harmful to natural soil fauna like Earth-worm. The study on the effect of the compound on matured seedlings revealed a negative effect. Thus, from the results of the present study, we can suggest that the active principle responsible for exerting the allelopathic effect in *Garcinia gummi-gutta* are flavonoid compound which can be exploited for the production of an environment friendly selective herbicide.

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LIST OF PUBLICATIONS

1. Dhanya.P., Benny P.J. 2013. Antifungal Effect of Methanolic Extracts of Leaves of *Garcinia Gummi-Gutta.L. Int. J. Pharm. Sci. Rev. Res*., 21(2).

2. Dhanya P, Benny PJ,. 2013.Allelopathy in *Solanum torvum* on selected crop plants found in India. *Journal of Global Pharma Technology*, 05(5):05-08 .

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